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Bifendate-chalcone hybrids: A new class of potential dual inhibitors of P-glycoprotein and breast cancer resistance protein

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ABSTRACT

We previously described bifendate-chalcone hybrids as potent P-glycoprotein inhibitors. In the present work, we determine whether these compounds could reverse breast cancer resistance protein (BCRP, ABCG2)-mediated multidrug resistance using HEK293/BCRP cells which was BCRP-transfected stable HEK293 cells. Results indicated that compounds **8d**, **8f**, **8g** and **8h** could significantly enhance mitoxantrone accumulation in HEK293/BCRP cells via inhibiting BCRP drug efflux function. The most active compound **8g** exhibited little intrinsic cytotoxicity (IC_{50} > 100 μM), and could reverse BCRP-mediated drug resistance independent of decreasing BCRP expression level. Notably, **8g** had little inhibitory effect on multidrug resistance-associated protein 1 (MRP1, ABCC1), another drug efflux transporter. The present findings, together with the previous results, suggest that **8g** might be act as dual inhibitors of P-gp and BCRP.

1. Introduction

Many cancers fail to respond to chemotherapy by acquiring multidrug resistance (MDR) during the treatment [1]. Studies have shown that MDR constituted a critical hurdle to cancer treatment, and has become one of the major causes of chemotherapy failure [2,3]. Although the mechanisms underlying MDR are complicated, the most common is overexpression of ATP binding cassette (ABC) transporters [4]. So far, 48 ABC transporters have been identified in humans, among them, P-glycoprotein (P-gp, ABCB1), breast cancer resistance protein (BCRP, ABCG2) and multidrug resistance-associated protein 1 (MRP1, ABCC1) are the best known and most important drug transporters of MDR, and could confer resistance to a broad spectrum of chemotherapeutic agents by exporting drugs out of cancer cells using energy from ATP hydrolysis [5,6].

An attractive strategy to overcome ABC transporter-mediated drug efflux is to develop inhibitors to sensitize cancer cells to chemotherapeutic drugs [7,8]. Generally, in order to preclude side effects it would be useful to inhibit only one transporter. However, P-gp, BCRP and/or MRP1, the prominent members of the ABC transporter superfamily, exhibited broad and partly overlapping substrate specificities [9], and sometimes could be detected in drug resistant cancer cells simultaneously. Under this situation, it might be beneficial to inhibit these transporters with a single compound [10]. For instance, overexpression of P-gp and BCRP is implicated in drug resistance of tyrosine kinase inhibitors (TKIs) [11], which were widely used in the treatment of cancer in clinic. Several studies have proven that TKIs were substrates of P-gp and BCRP [12], indicating that TKIs might be inactive and limited in treatment of patients with tumors overexpressing P-gp and BCRP, such as brain tumors [13]. In this regard, it is necessary to develop novel agents that could inhibit P-gp and BCRP simultaneously.

Our recently studies showed that a series of bifendate derivatives (**8a–i**), named as bifendate-chalcone hybrids (Table 1), could reverse P-gp mediated MDR more potently than verapamil (VRP) by inhibiting P-gp efflux function, and had little intrinsic cytotoxicity [14]. Literature survey revealed that chalcone, bioprecursor of flavonoids, possesses multiple bioactivities, such as antioxidation, anti-inflammation and anti-cancer [15,16]. Several studies have showed that chalcone could act as P-gp and/or BCRP inhibitors, thus reversing P-gp and/or BCRP mediated-MDR [17–19]. Based on these researches, we investigated whether these compounds could effectively reverse BCRP-mediated MDR in the current study. Additionally, their inhibitory effect on MRP1, another important ABC transporter, was also measured in this work.

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2. Materials and methods

2.1. Chemicals and reagents

Bifendate-chalcone hybrids (**8a–i**) were synthesized as previously described [14]. The chemical structure showed in Table 1. All electrophoresis reagents, protein concentration assay kit, and polyvinylidene difluoride membranes were purchased from Bio-Rad. Adriamycin and mitoxantrone were from Zhejiang Hisun Pharmaceutical Co., Ltd. and Jiangsu Hansoh Pharmaceutical Co., Ltd., respectively. Rhodamine 123, Ko143 and SRB were obtained from Sigma-Aldrich. Monoclonal antibody BXP-21 against ABCG2 was purchased from Abcam. MRPr1 against ABCC1 was from ARP American Research Products, Real-time PCR Master Mix was purchased from TOYOBO. Dimethyl sulfoxide (DMSO), TRIzol, and G418 were purchased from Invitrogen. Cell culture medium RPMI-1640 and Dulbecco's Modified Eagle's Medium were purchased from HyClone. All other chemicals were obtained from commercial sources of analytical grade.

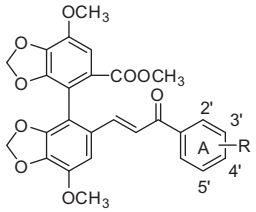
2.2. Cell lines and cell culture

HEK293 transfected sublines HEK293/Vec [20], HEK293/ABCC1 [21], and HEK293/ABCG2 [22] were obtained from Prof. Jian-ting Zhang (IU Simon Cancer Center, IN, USA), and cultured as previously described and frozen into multiple aliquots. All cells were passaged for 4 months or less before the renewal from frozen, early-passage stocks. All cell lines were periodically authenticated by morphologic inspection.

2.3. Cytotoxicity assay

The cytotoxicity was determined using the sulforhodamine B (SRB) colorimetric assay [23]. Briefly, the cells were seeded in 96-well plate at 3000 per well and cultured for 24 h. Adriamycin and mitoxantrone were then added to the cells, and the cells were cultured continuously for 3 days before the SRB assay. After the culture medium was aspirated, the cells were fixed and stained by adding 70 μL 0.4% (w/v) SRB in 1% acetic acid solution to each well and incubation at room temperature for 20 min. The plates were then washed three to five times with 150 μL of 1% acetic acid to remove the unbound SRB and air-dried. The bound SRB was then solubilized with 200 μL of 10 mmol/L unbuffered Tris-base, and the A_{570} nm was determined using a microplate reader (Thermo, USA). The effect of compound on drug resistance was determined by exposing cells to anticancer drugs in the absence or presence of different concentrations of the target compounds. And then the cytotoxicity was determined using SRB assay mentioned above.

Table 1
Chemical structures of target compounds.



Compound	R substituents: A-ring			
	2′	3′	4′	5′
8a	H	H	H	H
8b	H	H	OH	H
8c	H	H	OCH_3	H
8d	OCH_3	H	H	H
8e	OCH_3	H	OCH_3	H
8f	OCH_3	H	H	OCH_3
8g	CH_3	H	H	CH_3
8h	H	OCH_3	OCH_3	OCH_3
8i	H	H	NO_2	H

2.4. Flow cytometric analyses

1×10^6 cells in culture were pre-incubated with different concentrations of the target compounds, Ko143 or vehicle control (0.1% DMSO) for 1 h at 37 °C, followed by addition of 20 μmol/L mitoxantrone (MX) and incubation for 30 min, respectively. The reaction was stopped by addition of ice-cold PBS and centrifugation, washed with ice-cold PBS three times, and subjected analysis by flow cytometry. The relative values were identified by dividing the fluorescence intensity of each measurement by that of vehicle control.

2.5. Western blot analysis

Western blot analysis was done as previously described [20]. Briefly, cells were incubated with 5 μM individual compound or vehicle control for 48 h, respectively. Following incubation, the cells were harvested and lyzed. The cell lysates were separated by SDS–PAGE (12% gel) and transferred to a PVDF membrane. The blot was then probed with primary antibody followed by reaction with horseradish peroxidase-conjugated secondary antibody. After additional washes with TBST buffer, the protein-antibody complex were visualized by the enhanced Phototope TM-HRP Detection Kit (Cell Signaling, USA) and exposed to Kodak medical X-ray processor (Kodak, USA).

2.6. Reverse transcription-PCR

RNA extraction and real-time reverse transcriptase PCR (RT-PCR) were carried out as previously described [24]. The sequences of ABCG2 primers are 5′-TCATCAGCCTCGATATTCCA TCT-3′ (forward) and 5′-GGCCCGTGGAACATAAGTCTT-3′ (reverse). The sequences of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers are 5′-CCGTCTAGAAAAACCT GCC-3′ (forward) and 5′-GCCAAATTCGTTGTCATACC-3′ (reverse). The standard curve and data analysis were produced using Bio-Rad iQ5 software. The relative ABCG2 mRNA level treated with inhibitors was expressed as fold change of the control (in the presence of 0.1% DMSO).

2.7. Statistical analysis

Differences of the parameters between two cell groups were analyzed by two-tailed Student's t test. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Effect of bifendate-chalcone hybrids on mitoxantone accumulation in HEK293/BCRP and HEK293 cells

As mentioned above, drugs (substrates of BCRP) could be pumped out of the resistant cells overexpressing BCRP, resulting in a decrease of the intracellular accumulation. When the efflux function of BCRP was inhibited by a BCRP inhibitor, drug accumulation level in the cells might be increased [25]. Therefore, the intracellular mitoxantrone (MX, a model substrate of BCRP) accumulation in BCRP-transfected stable HEK293/BCRP cells and their corresponding BCRP vector transfected HEK293/VEC cells were measured in the presence or absence of **8a–i** (5 μM, nontoxic dose)

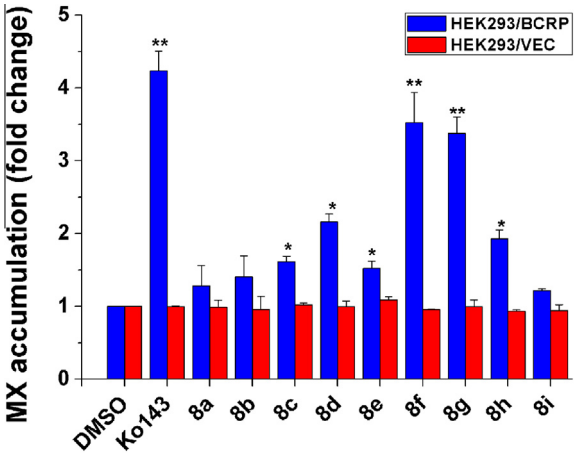


Fig. 1. Mitoxantrone (MX) accumulation in HEK293/VEC and HEK293/BCRP cells treated with target compounds and Ko143 (5 μM), respectively. Data are expressed as mean values ± SD, $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* DMSO group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by flow cytometric analysis, and a BCRP specific inhibitor Ko143 was selected as the positive control. As can be seen in Fig. 1, most of the target compounds could slightly raise the accumulation level of MX in HEK293/BCRP cells (Fig. 1, blue bar), indicating that the efflux function of BCRP might be inhibited by the target compounds. Among them, **8f** and **8g** displayed the best potency, which was almost comparable with the BCRP inhibitor Ko143. Notably, all the compounds have no effect on MX accumulation in BCRP-negative HEK293/VEC cells (Fig. 1, red bar), suggesting that the effect of these compounds on MX accumulation is likely via inhibiting BCRP.

3.2. The cytotoxicity of active compounds

Since an ideal BCRP inhibitor should reverse MDR at non-toxic concentrations [26], the cytotoxicity of the most active compounds **8d**, **8f**, **8g** and **8h** against HEK293/BCRP and their corresponding BCRP-negative HEK293/VEC cells was determined by SRB assay. Anticancer drug MX was served as the positive control. As shown in Table 2, HEK293/BCRP cells were obviously resistant to MX. Its IC_{50} value (1.102 μM) was 91-fold higher than that of HEK293/VEC cells (0.01208 μM). It is noteworthy that the active compounds exhibited little ($IC_{50} > 100$ μM) or slight ($IC_{50} = 20.36$–37.47 μM) intrinsic cytotoxicity in vitro, indicating that this kind of compounds was suitable to the development of BCRP inhibitors with safety profile.

3.3. Reversal effect of active compounds on BCRP-mediated drug resistance

Subsequently, we determined whether these compounds could reverse BCRP-mediated drug resistance at non-toxic concentrations ($<IC_{10}$) according to a previously described method with minor modification [24]. Briefly, the cytotoxicity of anticancer drug MX on HEK293/BCRP and HEK293/VEC cells was evaluated in the presence or absence of **8d**, **8f**, **8g** and **8h** at various concentrations (CH: 5.0 μM, CM: 2.5 μM and CL: 1.25 μM) by SRB assay, and the BCRP inhibitor Ko143 was employed as the positive control. As shown in Fig. 2, due to drug resistance, anticancer drug MX displayed little inhibitory effect on the proliferation of HEK293/BCRP cells (Fig. 2A, blue bar). However, the cytotoxicity of MX was dramatically increased when combination with **8d**, **8f**, **8g** and **8h** at non-toxic concentrations (CH: 5.0 μM, CM: 2.5 μM and CL: 1.25 μM), indicating that these compounds could restore HEK293/BCRP cells sensitivity to MX. Obviously, **8g** showed more potent chemo-sensitizing effect than the others at the high concentration (Fig. 2, yellow bar), for the survival rate of HEK293/BCRP cells treated with **8g** was the lowest (30%), which displayed almost identical potency with that of the classical BCRP inhibitor Ko143 (29%). Interestingly, such chemo-sensitizing effect was not observed in BCRP-negative HEK293/VEC cells (Fig. 2B), suggesting that the chemo-sensitizing effect was achieved via inhibiting BCRP.

Table 2
IC_{50} values of target compounds against HEK293/BCRP and HEK293/VEC cells.

Compound	IC_{50} (μM)[a]	
	HEK293/BCRP	HEK293/VEC
8d	37.47	36.83
8f	>100	>100
8g	>100	>100
8h	20.36	23.47
MX	1.102	0.01208

[a] IC_{50} values are expressed as means of triplicate experiments.

3.4. The effect of active compounds on mRNA and protein expression levels of BCRP

It is believed that ABC transporters-mediated MDR can be reversed either by inhibiting transporters' function and/or decreasing their expression level [27], we next determined whether the active compounds **8d**, **8f**, **8g** and **8h** could affect mRNA and protein expression levels of BCRP using RT-PCR and Western blot, respectively [25]. As compared to vehicle (0.1% DMSO), both the mRNA and protein levels of BCRP in HEK293/BCRP cells had no obviously changes when treated with **8d**, **8f**, **8g** and **8h** (5 μM) for 48 h (Fig. 3), suggesting that the MDR reversal activity of the target compounds was not achieved by decreasing BCRP expression level, but by inhibiting BCRP efflux function.

3.5. Effect of **8g** on function and sensitization of MRP1

Finally, in order to assess the selectivity towards P-gp and BCRP, we further screened the reversal activity of the most active compound **8g** on MRP1-mediated MDR in MRP1-transfected stable HEK293 cells (HEK293/MRP1). Results showed that **8g** could not increase adriamycin (ADR, a MRP1 substrate) accumulation in HEK293/MRP1 cells (Fig. 4A), or sensitize HEK293/MRP1 cells to ADR (Fig. 4B), suggesting that **8g** could not reverse MRP1-mediated MDR.

4. Discussion

Multidrug resistance (MDR) phenotype is regarded as the major obstacle to successful chemotherapy in cancer patients [28]. Although MDR involves multiple mechanisms, the primary mechanism is the overexpression of ATP-binding cassette (ABC) transporters in the plasma membrane of drug resistant cells [29]. Among them, the best known and most extensively studied are P-gp, BCRP and MRP1. An attractive strategy to overcome MDR is to develop ABC transporter inhibitors to sensitize cancer cells to anticancer drugs [7,8]. Recently, we synthesized a series of novel bifendate-chalcone hybrids (**8a–i**) as P-gp inhibitors [14]. Preliminary screening results showed that most of the target compounds exhibited strong P-gp inhibitory effect, and compounds **8g** could more potently reversed P-gp-mediated MDR than the classical P-gp inhibitor VRP by inhibiting P-gp efflux function. Additionally, unlike VRP, **8g** showed no stimulation of the P-gp ATPase activity,

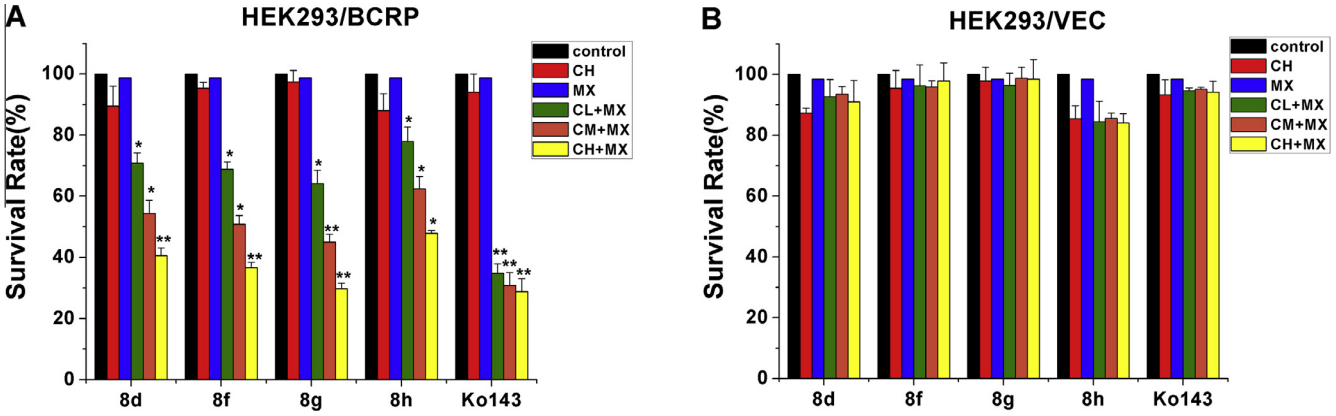


Fig. 2. Chemo-sensitizing effect of the target compounds. The cytotoxicity of MX against HEK293/BCRP cells (A) and HEK293/VEC cells (B) in the presence or absence of the target compounds at various concentrations (CH: 5.0 μM; CM: 2.5 μM; CL: 1.25 μM) was measured by SRB assay. The BCRP inhibitor Ko143 was selected as a positive control. Survival rate was expressed as percentage mean of cell growth ± S.D. with respect to the control without any treatment of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* MX treatment alone group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

indicating that it was not a P-gp substrate. Besides, the intrinsic cytotoxicity of **8g** was very low (IC_{50} > 200 μM) in vitro, therefore, **8g** might represent a promising lead to develop P-gp-mediated MDR reversal agents in cancer chemotherapy. Notably, recent reports have revealed that several chalcone derivates could inhibit not only P-gp but also other ABC transporters such as BCRP and MRP1, thus reversing P-gp, BCRP and/or MRP1 mediated-MDR [17–19]. Based on these researches, we speculated that bifendate-chalcone hybrids might inhibit three major ABC transporters. Therefore, we investigated whether these compounds could effectively reverse BCRP and/or MRP1-mediated MDR in the present study.

We firstly investigate the inhibitory effect of target compounds on BCRP by flow cytometric analysis. Our results showed that most of the target compounds could increase intracellular accumulation of MX in HEK293/BCRP cells, and displayed little effect on MX accumulation in BCRP-negative HEK293/VEC cells, indicating that these bifendate-chalcone hybrids might increase the cellular concentration of anti-cancer drug by interfering BCRP.

Since an ideal ABC transporter inhibitor should reverse MDR at non-toxic concentrations, we next examined the innate cytotoxicity of the most active compounds **8d**, **8f**, **8g** and **8h** against HEK293/BCRP and their corresponding BCRP-negative HEK293/VEC cells by SRB assay. Encouragingly, we found that the active compounds displayed little intrinsic cytotoxicity in vitro. These results indicated that this kind of compounds might be suitable to develop safe BCRP inhibitors.

With the data obtained, we subsequently determined whether these compounds could restore HEK293/BCRP cells sensitivity to anticancer drug MX at non-toxic concentrations. It was seen that due to drug resistance MX exhibited little effect on the proliferation of HEK293/BCRP cells, however, the cytotoxicity of MX was dramatically increased when combination with **8d**, **8f**, **8g** and **8h** at non-toxic concentrations. Notably, **8g** displayed almost identical chemo-sensitizing potency with that of the classical BCRP inhibitor Ko143 at the high concentration. Moreover, such effect was not observed in BCRP-negative HEK293/VEC cells. Subsequently, we found that the active compounds **8d**, **8f**, **8g** and **8h** had little effect on BCRP expression at mRNA and protein levels in HEK293/BCRP cells. These results indicated that the MDR reversal activity of the active compounds was not achieved by decreasing BCRP expression level, but by inhibiting BCRP function.

Finally, we further examined the inhibitory effect of the most active compound **8g** on MRP1. Data showed that **8g** could neither increase intracellular accumulation of adriamycin (a MRP1 substrate) in HEK293/MRP1, nor restore HEK293/MRP1 cells sensitivity to anticancer drug ADR, suggesting that **8g** could not reverse MRP1-mediated MDR.

In conclusion, nine bifendate-chalcone P-gp inhibitors (**8a**–**i**) were assessed in vitro as reversal agents of BCRP. Cytotoxicity evaluation revealed that the most active compound **8g** exhibited little intrinsic cytotoxicity in vitro, and could increase the intracellular accumulation of the substrate anticancer drug by inhibiting BCRP efflux function, thus reversing BCRP-mediated MDR independent

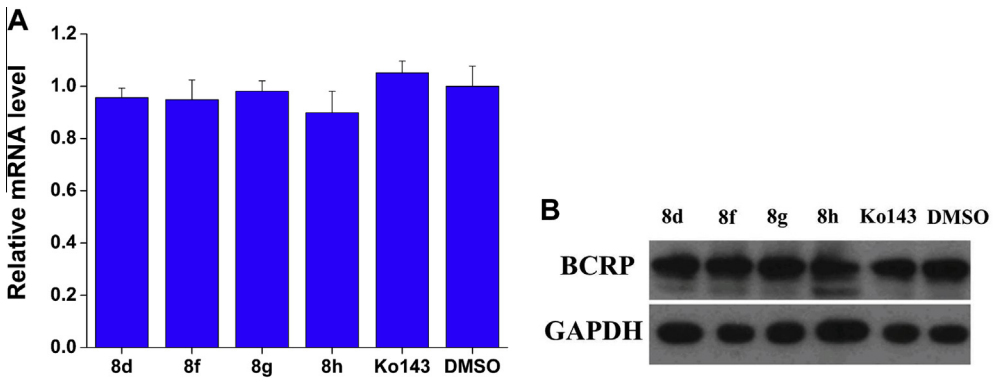


Fig. 3. Effects of **8d**, **8f**, **8g** and **8h** on BCRP mRNA and protein expression level. (A) The relative BCRP mRNA level treated with target compounds was expressed as fold change of the control cultures (in the presence of 0.1% DMSO). Data shown are mean ± S.D. from three independent experiments. (B) Effect of target compounds on BCRP expression at protein level. Independent experiments were performed at least three times, and a representative experiment is shown.

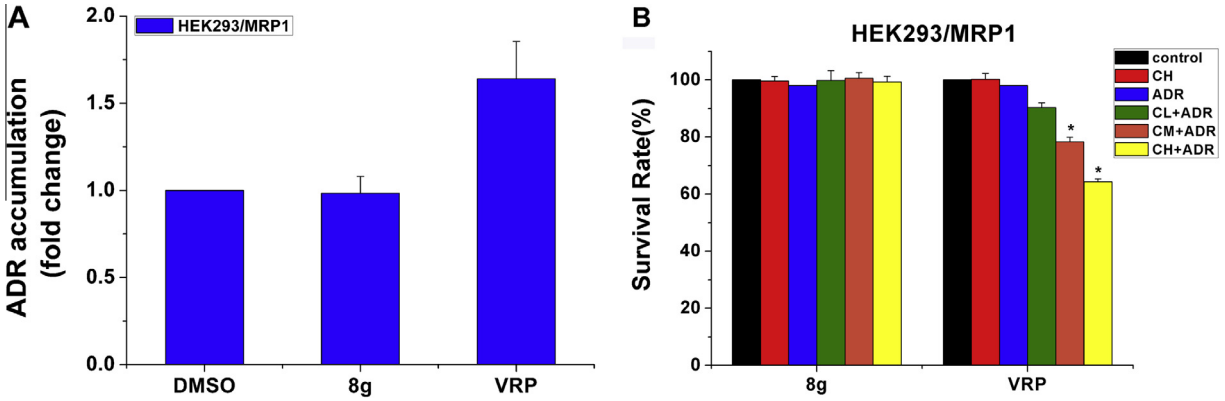


Fig. 4. Effect of **8g** on the function and sensitization of MRP1. (A) ADR accumulation in HEK293/MRP1 cells treat with **8g** (5 μM). VRP (10 μM) was served as a positive control. Data are expressed as mean values ± SD, $n = 3$. (B) The cytotoxicity of ADR against HEK293/MRP1 cells in the presence or absence of **8g** (CH: 5 μM; CM: 2.5 μM; CL: 1.25 μM) was evaluated by SRB assay. VRP (CH: 10 μM; CM: 5 μM; CL: 2.5 μM) was selected as a positive control. Survival rate was expressed as percentage mean of cell growth ± S.D. with respect to the control without any treatment of three independent experiments. $^{*}P < 0.05$ *vs.* ADR treatment alone group.

of decreasing BCRP expression level. Notably, **8g** displayed little reversal effect on MRP1-mediated drug resistance. The present findings, together with the previous results, suggest that **8g** might be act as dual inhibitors of P-gp and BCRP to warrant further evaluation.

Acknowledgments

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References

[1] S.G. Aller, J. Yu, A. Ward, Y. Weng, S. Chittaboina, R. Zhuo, P.M. Harrell, Y.T. Trinh, Q. Zhang, I.L. Urbatsch, G. Chang, Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding, Science 323 (2009) 1718–1722.
[2] M. Saraswathy, S. Gong, Different strategies to overcome multidrug resistance in cancer, Biotechnol. Adv. 31 (2013) 1397–1407.
[3] S.A. Santos, A. Paulo, Small molecule inhibitors of multidrug resistance gene (MDR1) expression: preclinical evaluation and mechanisms of action, Curr. Cancer Drug Targets 13 (2013) 814–828.
[4] Y.H. Choi, A.M. Yu, ABC transporters in multidrug resistance and pharmacokinetics, and strategies for drug development, Curr. Pharm. Des. 20 (2014) 793–807.
[5] E. Crowley, C.A. McDevitt, R. Callaghan, Generating inhibitors of P-glycoprotein: where to, now?, Methods Mol Biol. 596 (2010) 405–432.
[6] J.P. Gillet, M.M. Gottesman, Advances in the molecular detection of ABC transporters involved in multidrug resistance in cancer, Curr. Pharm. Biotechnol. 12 (2011) 686–692.
[7] C. Martelli, M. Coronnello, S. Dei, D. Manetti, F. Orlandi, S. Scapecchi, M. Novella Romanelli, M. Salerno, E. Mini, E. Teodori, Structure–activity relationships studies in a series of N, N-bis(alkanol)amine aryl esters as P-glycoprotein (Pgp) dependent multidrug resistance (MDR) inhibitors, J. Med. Chem. 53 (2010) 1755–1762.
[8] C. Martelli, D. Alderighi, M. Coronnello, S. Dei, M. Frosini, B. Le Bozec, D. Manetti, A. Neri, M.N. Romanelli, M. Salerno, S. Scapecchi, E. Mini, G. Sgaragli, E. Teodori, N, N-bis(cyclohexanol)amine aryl esters: a new class of highly potent transporter-dependent multidrug resistance inhibitors, J. Med. Chem. 52 (2009) 807–817.
[9] J.S. Lagas, M.L. Vlaming, A.H. Schinkel, Pharmacokinetic assessment of multiple ATP-binding cassette transporters: the power of combination knockout mice, Mol. Interv. 9 (2009) 136–145.
[10] S.P. Ebert, B. Wetzel, R.L. Myette, G. Conseil, S.P. Cole, G.A. Sawada, T.W. Loo, M.C. Bartlett, D.M. Clarke, M.R. Detty, Chalcogenopyrylium compounds as modulators of the ATP-binding cassette transporters P-glycoprotein (P-gp/ABCB1) and multidrug resistance protein 1 (MRP1/ABCC1), J. Med. Chem. 55 (2012) 4683–4699.
[11] A. Brozik, C. Hegedus, Z. Erdei, T. Hegedus, C. Ozvegy-Laczka, G. Szakacs, B. Sarkadi, Tyrosine kinase inhibitors as modulators of ATP binding cassette multidrug transporters: substrates, chemosensitizers or inducers of acquired multidrug resistance?, Expert Opin Drug Metab. Toxicol. 7 (2011) 623–642.
[12] S. Agarwal, R. Sane, J.L. Gallardo, J.R. Ohlfest, W.F. Elmquist, Distribution of gefitinib to the brain is limited by P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2)-mediated active efflux, J. Pharmacol. Exp. Ther. 334 (2010) 147–155.
[13] S. Agarwal, A.M. Hartz, W.F. Elmquist, B. Bauer, Breast cancer resistance protein and P-glycoprotein in brain cancer: two gatekeepers team up, Curr. Pharm. Des. 17 (2011) 2793–2802.
[14] X. Gu, Z. Ren, X. Tang, H. Peng, Y. Ma, Y. Lai, S. Peng, Y. Zhang, Synthesis and biological evaluation of bifendate-chalcone hybrids as a new class of potential P-glycoprotein inhibitors, Bioorg. Med. Chem. 20 (2012) 2540–2548.
[15] M.R. El Sayed Aly, H.H. Abd El Razek Fodah, S.Y. Saleh, Antiobesity, antioxidant and cytotoxicity activities of newly synthesized chalcone derivatives and their metal complexes, Eur. J. Med. Chem. 76 (2014) 517–530.
[16] K.V. Sashidhara, M. Kumar, R.K. Modukuri, R. Sonkar, G. Bhatia, A.K. Khanna, S. Rai, R. Shukla, Synthesis and anti-inflammatory activity of novel biscoumarin-chalcone hybrids, Bioorg. Med. Chem. Lett. 21 (2011) 4480–4484.
[17] L.P. Rangel, E. Winter, C. Gauthier, R. Terreux, L.D. Chiaradia-Delatorre, A. Mascarello, R.J. Nunes, R.A. Yunes, T.B. Creczynski-Pasa, S. Macalou, D. Lorendeau, H. Baubichon-Cortay, A. Ferreira-Pereira, A. Di Pietro, New structure–activity relationships of chalcone inhibitors of breast cancer resistance protein: polyspecificity toward inhibition and critical substitutions against cytotoxicity, Drug Des. Devel. Ther. 7 (2013) 1043–1052.
[18] K. Juvale, K. Stefan, M. Wiese, Synthesis and biological evaluation of flavones and benzoflavones as inhibitors of BCRP/ABCG2, Eur. J. Med. Chem. 67 (2013) 115–126.
[19] Z. Parveen, G. Brunhofer, I. Jabeen, T. Erker, P. Chiba, G.F. Ecker, Synthesis, biological evaluation and 3D-QSAR studies of new chalcone derivatives as inhibitors of human P-glycoprotein, Bioorg. Med. Chem. 22 (2014) 2311–2319.
[20] J. Xu, H. Peng, Q. Chen, Y. Liu, Z. Dong, J.T. Zhang, Oligomerization domain of the multidrug resistance-associated transporter ABCG2 and its dominant inhibitory activity, Cancer Res. 67 (2007) 4373–4381.
[21] Y. Yang, Q. Chen, J.T. Zhang, Structural and functional consequences of mutating cysteine residues in the amino terminus of human multidrug resistance-associated protein 1, J. Biol. Chem. 277 (2002) 44268–44277.
[22] J. Xu, Y. Liu, Y. Yang, S. Bates, J.T. Zhang, Characterization of oligomeric human half-ABC transporter ATP-binding cassette G2, J. Biol. Chem. 279 (2004) 19781–19789.
[23] H. Peng, J. Qi, Z. Dong, J.T. Zhang, Dynamic vs static ABCG2 inhibitors to sensitize drug resistant cancer cells, PLoS One 5 (2010) e15276.
[24] H. Peng, Z. Dong, J. Qi, Y. Yang, Y. Liu, Z. Li, J. Xu, J.T. Zhang, A novel two mode-acting inhibitor of ABCG2-mediated multidrug transport and resistance in cancer chemotherapy, PLoS One 4 (2009) e5676.
[25] Y. Wei, Y. Ma, Q. Zhao, Z. Ren, Y. Li, T. Hou, H. Peng, New use for an old drug: inhibiting ABCG2 with sorafenib, Mol. Cancer Ther. 11 (2012) 1693–1702.
[26] S. Shukla, C.P. Wu, S.V. Ambudkar, Development of inhibitors of ATP-binding cassette drug transporters: present status and challenges, Expert Opin. Drug Metab. Toxicol. 4 (2008) 205–223.
[27] C.L. Dai, A.K. Tiwari, C.P. Wu, X.D. Su, S.R. Wang, D.G. Liu, C.R. Ashby Jr., Y. Huang, R.W. Robey, Y.J. Liang, L.M. Chen, C.J. Shi, S.V. Ambudkar, Z.S. Chen, L.W. Fu, Lapatinib (Tykerb, GW572016) reverses multidrug resistance in cancer cells by inhibiting the activity of ATP-binding cassette subfamily B member 1 and G member 2, Cancer Res. 68 (2008) 7905–7914.
[28] P.D. Eckford, F.J. Sharom, ABC efflux pump-based resistance to chemotherapy drugs, Chem. Rev. 109 (2009) 2989–3011.
[29] D. Turk, M.D. Hall, B.F. Chu, J.A. Ludwig, H.M. Fales, M.M. Gottesman, G. Szakacs, Identification of compounds selectively killing multidrug-resistant cancer cells, Cancer Res. 69 (2009) 8293–8301.